

The DEBRA International Visioning/Consensus Meeting on Epidermolysis Bullosa: Summary and Recommendations

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EPIDERMOLYSIS BULLOSA (EB): THE CLINICAL PERSPECTIVE

EB is a group of heritable skin disorders manifesting with blistering and erosions as a result of trauma to the skin. The disease is both genetically and clinically heterogeneous, and adding to the clinical complexity are the extracutaneous manifestations, including gastrointestinal, respiratory, and vesicourinary tract involvement, as well as manifestations in the hair, nails, and teeth. The spectrum of the clinical severity is highly variable: the milder forms of the disease are characterized by life-long blistering, primarily of the hands and feet, whereas the most severe forms can lead to the demise of the affected individual at birth or shortly thereafter.

Depending on the level of tissue separation, as determined by transmission electron microscopy or immunoepitope mapping of the basement membrane zone (BMZ), EB has been traditionally divided into three broad categories: (i) the simplex forms are characterized by fragility of the basal keratinocytes; (ii) the junctional forms depict tissue separation within the lamina lucida; and (iii) the dystrophic forms are characterized by tissue cleavage below the lamina densa on the dermal side of the cutaneous basement membrane zone at the level of the anchoring fibrils.

The various forms of EB present with a formidable clinical challenge primarily due to the unrelenting nature of the skin involvement, manifesting with continuous blistering, erosions, chronic ulceration, and scarring. In certain forms of the disease, early epidermal metaplasia can lead to the development of aggressive squamous cell carcinomas which may necessitate amputation of the limbs, and frequently lead to premature demise of the affected individual.

THE PROGRESS OF MOLECULAR GENETICS IN EB

Over the past decade, tremendous progress has been made in understanding the molecular basis of different variants of EB. In fact, specific mutations have now been identified in 10 distinct genes expressed in the cutaneous BMZ. In normal skin, the products of such genes form an intricate network structure which spans the dermal-epidermal BMZ extending from the cytoplasmic milieu of the basal keratinocytes on the epidermal side to the underlying dermal mesenchyme. Genetic defects in these structural components of the BMZ, which result in either absent expression of the gene or synthesis of a functionally altered protein, lead to instability of the dermal-epidermal junction, manifesting with fragility of the skin. The nature and the combination of the specific mutations, superimposed on the individuals' genetic background, explain the clinical variability of EB in different families.

The progress in molecular genetics of EB has resulted in several major clinical applications. It has led to improvements in diagnosis, genetic counseling and prognosis. It has also provided the basis for

DNA-based prenatal testing and preimplantation genetic diagnosis of EB. Finally, this progress is a prerequisite for the development of gene therapy approaches for this group of devastating skin diseases.

THE VISIONING/CONSENSUS MEETING ON EB

Despite the impressive advances in our understanding of the molecular defects in EB, there is still no specific therapy for any form of the disease. This challenge brought a number of leading investigators in the field, from the United States, Europe, and Japan, together on October 22–24, 1999, in New Jersey, U.S.A. This visioning/consensus meeting was a joint initiative of the Dystrophic Epidermolysis Bullosa Research Associations (DEBRA) of America and the U.K., the leading patient advocacy organizations on both sides of the Atlantic and members of the DEBRA International, the Coalition of DEBRA in 29 countries. The purpose of the meeting was to identify carefully the obstacles and challenges, from the vantage point of the advances over the past decade, that have hampered further progress towards rational treatment and cure of EB. The three clinical areas emphasized in this meeting dealt with compromised wound healing, the problem of carcinogenesis, and the challenge of gene therapy. The overall goals of this meeting were to build a consensus and to develop a unifying blueprint through collaborative efforts towards improved understanding and treatment of EB. The process was assisted by outside facilitators, scientists with expertise in the areas to be considered but not directly involved in EB research.

The stage of the meeting was set by Dr. Ronald Crystal (Weill Medical College of Cornell University, New York) as the keynote speaker. Dr. Crystal highlighted the current status of gene therapy trials both in the United States and Europe, and reviewed the pros and cons of various options currently available for gene therapy in general. He emphasized that the reality of the clinical gene therapy in fact follows the concept of a successful drug development, requiring meticulous refinement of individual steps and consideration of alternative approaches. He advocated the launch of a limited human gene therapy trial on EB without extensive animal work, as "animal models may not predict what's happening in humans".

Dr. Lorne Taichman (SUNY Stony Brook, New York) addressed issues more directly relevant to the epidermal gene therapy by reviewing the recent progress made in the retroviral delivery of genes to keratinocytes. He recognized appropriate animal models as targets of initial gene therapy, development of both *ex vivo* and *in vivo* gene transfer, and grafting of epidermal keratinocytes as major challenges for cutaneous gene therapy.

Following these presentations, in-depth discussions of the three emphasis areas of EB, namely, wound healing, carcinogenesis, and gene therapy, were conducted.

ISSUES OF COMPROMISED WOUND HEALING

The challenge of compromised wound healing due to repetitive trauma to the skin, the defining clinical feature of this condition, is

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a major problem encountered in patients with EB. In fact, development of novel therapies for EB aims at preventing the occurrence of chronic wounds and at enhancement of their healing by accelerating the naturally occurring processes at the cellular level. At the same time, wound healing has relevance to carcinogenesis in EB, as the skin cancers in these patients develop primarily at the site of chronically damaged skin. Finally, some of the techniques being considered for treatment of compromised wound healing may have relevance to gene therapy for EB, e.g., the growth and grafting of skin cells could be used as a system to deliver genetic material to the skin.

In the discussion led by Dr. Jeffrey Morgan (Shriners Hospital for Children, Boston, MA) it was agreed that an understanding of the molecular and cellular events of wound healing is critical to the design of new therapies, both genetic as well as epigenetic, for the treatment and management of wounds associated with various forms of EB. The healing of an acute primary wound progresses through a series of fairly well defined stages of temporal events which lead to closure and remodeling of the wound. In contrast, the wounds in EB are not phenotypically uniform, and they represent different stages of acute and chronic wounds, manifesting with granulation tissue, impaired re-epithelialization, and scarring. The wound healing processes in EB are also complicated by infections, malnutrition, and immunologic suppression of the host, and the standard of care of such wounds has not been established.

It was agreed that studies are needed to define the basic characteristics of the wound healing phases in EB and to see how they differ from normal wound healing processes. Towards understanding the basic biology of wound healing in EB, studies attempting to profile the influence of various forms of this disease would include assessment of the following processes: cell migration and re-epithelialization; the proteolytic environment of the wounds; the kinetics and temporal sequence of the wound healing processes; the influence of the wound healing environment on the prospects of *in vivo* gene transfer; the cytokine profile of wound healing; the processes of wound remodeling and scar formation; the formation of the granulation tissue; the role of cell-cell and cell-matrix interactions; and the role of bacterial infections on the wound healing process. It was recognized that detailed knowledge of the basic biology and pathology of EB wounds is a prerequisite for the development of new strategies and technologies to manage the wounds associated with EB.

Of particular interest in the wound healing field relevant to EB is the recent development of skin substitutes by tissue engineering. This rapidly advancing field lacks, however, at this juncture carefully controlled comparative studies which would critically evaluate the suitability of the currently available skin substitute products for the acute and long-term treatment of EB, as well as their suitability to act perhaps as vehicles for *ex vivo* gene therapy. It was agreed that the immunologic aspects of allogeneic cell substitutes need to be carefully examined before their widespread use can be advocated for skin replacement in EB. It was also recognized that recombinant extracellular matrix proteins, as exemplified by type VII collagen and laminin 5, could influence wound healing processes when added exogenously. Furthermore, in light of the extensive scarring, particularly in the dystrophic forms of EB, novel anti-scarring technologies should be explored. It was emphasized that, irrespective of the treatment, the end-points of measurement of efficacy are not well developed, and the standard of care for EB wounds should be established. In general, the challenge of wound healing management in EB should attract new investigators and additional resources to the EB field and should benefit from cross-reference to the extensive work on wound healing in general and the field of tissue fibrosis in particular.

The discussion on wound healing identified the following areas of research particularly important in relation to EB.

1 Development of systematic, comprehensive approaches to identify factors characteristic of the biologic profile of compromised wound healing in EB through: (i) characterization of the processes

of activation/deactivation with respect to cell growth, migration, and differentiation, with reference to re-epithelialization and malignant transformation; (ii) assessment of the wound healing profile in EB by evaluation of cytokine expression with reference to the modulation of the extracellular matrix/protease gene expression and activation mechanisms; and (iii) evaluation of the role of wound healing environment in reference to cell-cell and cell-matrix interactions, and the role of immunologic and biologic modifiers.

2 Development of links to the mainstream wound healing research currently being performed outside of the EB research spheres either in academia or in industrial settings, to take advantage of the unique perspective offered by wound healing in EB.

3 Evaluation of new and emerging technologies for enhanced wound healing with emphasis on: (i) gene transfer therapies toward accelerated and corrective healing processes; (ii) assessment of the efficacy of bioengineered skin substitutes to promote compromised wound healing in EB in comparative clinical trials; (iii) examination of anti-scarring technologies by testing existing cytokines (transforming growth factor- β 3) or biologic compounds (mannose-6-phosphate) for their efficacy in limiting scarring toward cosmetic and functional improvement in patients with EB, and development of novel compounds for such purpose.

4 Development of technologies which would allow critical, reproducible, and clinically relevant assessment of the changes in the wound healing process for use in clinical trials.

THE PROBLEM OF CARCINOGENESIS IN EB

Carcinogenesis represents a major clinical problem for a subset of EB patients, often with catastrophic consequences. Specifically, it is well recognized that patients with recessive dystrophic EB (RDEB), in particular the Hallopeau-Siemens subtype, and to much lesser extent non-Herlitz junctional EB, are at risk for the development of one or more squamous cell carcinomas (SCC). Based on data collected by the National EB Registry in the United States and collaborators at St Thomas' Hospital, London, we now know that these tumors can arise in young adults, have a predilection for the extremities, and histologically usually appear well differentiated. It is also recognized that the development of multiple primary SCC is the rule in RDEB, that the cumulative risk of SCC rises nearly geometrically during successive 5 y increments, and that at least 50% of all RDEB patients will develop at least one SCC by age 40. These tumors behave aggressively, resulting in the death of most patients within 5 y of detection of their first SCC. The latter outcome occurs regardless of how widely excised these primaries are; metastases tend to be very poorly responsive to radiation treatment and to chemotherapy.

It is also recognized that EB patients at risk for SCC have other features which are likely to facilitate the development of such tumors, including (i) grossly impaired nutrition, secondary to chronic, severe gastrointestinal tract disease activity, which may indirectly impair host immunity, contributing to recurrent infections and altered wound healing, and (ii) altered *in vitro* cellular immunity against tumor cells (most pertinently, abnormal function of circulating natural killer cells), the latter of which may be associated with abnormal tumor cell surveillance *in vivo*. Finally, it is also unfortunately clear that little is otherwise known about the basic biology of SCC which arise in the setting of RDEB, and that there is as yet no proven means of chemoprevention.

On the basis of the considerations summarized above, discussion led by Dr. Douglas Lowy (NCI, NIH) identified the following areas of research as potentially fruitful in an attempt to find better cures, as well as some effective means of chemoprevention, of these particularly devastating tumors.

1 Determination of the underlying basic mechanism(s) by which SCC arise in EB (especially the RDEB), to include: (i) precise molecular characterization of these SCC, in particular as it pertains

to mutations in oncogenes or genes encoding for various tumor suppressor factors; (ii) ascertainment of the role, if any, of human papilloma virus infection in the promotion of these tumors—identification of human papilloma virus as a causative or contributing agent would suggest the possibility of vaccine therapy in children and adults with this disease; (iii) determination of how the chronic absence of type VII collagen along the cutaneous BMZ may help promote SCC transformation, growth, and/or spread; (iv) role of specific arms of chronic inflammatory pathways (cytokines; proteolytic enzymes, such as collagenase) in the promotion of SCC in RDEB patients; (v) the way by which chronic, abnormal wound healing contributes to the development of biologically aggressive SCC; and (vi) development of novel animal models which will permit more precise study on the relationship between type VII collagen mutations and the development of SCC.

2 Encouragement of studies to determine why these tumors are so biologically aggressive despite their frequent high level of differentiation histologically, to include why they have such a marked predilection for recurrence and metastasis (regional and distant) despite early, wide surgical excision with no evidence of residual tumor at any of the excised tissue margins.

3 Encouragement of design and execution of properly designed clinical trials specifically aimed at the development of: (i) novel, effective therapies (both primary and adjuvant) for the treatment of recurrent or metastatic disease; (ii) successful approaches to chemoprevention of these SCC; (iii) novel therapies which, in improving wound healing and/or reducing scar formation within targeted areas, will lead to a reduced risk of developing SCC or cancerous precursors in these sites.

4 Development of novel, noninvasive means for the accurate, early diagnosis of SCC *in vivo*.

THE CHALLENGE OF GENE THERAPY FOR EB

The breathtaking progress in delineating the molecular basis of various genodermatoses, including EB, has provided the prerequisite information for development of gene therapy strategies. At the same time, skin is thought to be a good target organ for gene therapy, as the constitutive cells of skin, such as epidermal keratinocytes and dermal fibroblasts, can be readily propagated and manipulated in culture, and regrafted to the skin. Furthermore, skin is directly accessible for *in vivo* transfer of genes, and for direct examination of the efficacy and safety of the therapy. Yet, cutaneous gene therapy is still not a reality, and the challenges are multiple.

The discussion, led by Dr. Peter Humphries (Trinity College, Dublin) and Dr. Lorne Taichman (SUNY Stony Brook, New York) concentrated on various challenges of gene therapy for EB. The discussion considered first various issues relating to the delivery of the transgene, including viral vectors and delivery by physical means (electroporation, slow release polymers, liposomes, etc.). It was pointed out that for each different mode of delivery, the risk/benefit ratio in terms of correction efficiency and site of integration (or lack of it) should be considered. Is the therapy to be limited to a small area of skin, or is more global correction of the skin feasible? Is repeated treatment of a localized area feasible towards improvement, such as maintenance of the functionality of hands in RDEB? Many of the issues relating to the safety (chance of mutagenesis, spreading to other tissues, germline transmission, etc.) are germane to gene therapy approaches in general, not necessarily limited to considerations in EB.

Of particular interest towards the treatment of dominant forms of EB is the development of two areas of gene therapy. The first relates to refinement of ribozyme technology, a strategy that can be built around polymorphisms associated with dominant mutations. The second promising area relates to the use of chimeric RNA-DNA oligomer technology for correction of single-base substitutions by homologous recombination. The latter technique has recently shown considerable promise both for *in vitro* and *in vivo* correction of point mutations in model systems, such as tyrosinase deficiency in albino melanocytes.

An intermediate step towards the development of gene therapy for humans could involve testing of such strategies in animal models. It was recognized that for different forms of EB, a variety of animal models mimicking the clinical, genetic, and ultrastructural features of this disease are available. Specifically, transgenic animals with targeted ablation of two of the three laminin 5 genes and type VII collagen, as well as of the $\alpha 6$ and $\beta 4$ integrin genes, are available. In addition, a number of naturally occurring animal models of EB, including sheep, cats, and dogs, have been identified. It was also noted that development of additional animal models, particularly with technologies allowing temporally conditional or tissue-specific ablation of the target genes, will be helpful for further testing of gene therapy strategies.

Considerable discussion centered on the importance of a demonstration project (“proof of principle”) for a gene therapy trial. Some participants felt that such limited efficacy trials are a necessary intermediate step prior to more widespread testing of the genetic therapeutics, whereas others agreed that trials in animal models might obviate this intermediate step. Nevertheless, a consensus was reached that a clinical demonstration project to validate a gene therapy approach would be advantageous. Such a trial would demonstrate that gene transfer is capable of phenotypic correction which, in turn, would accelerate the overall development of novel genetic-based therapies. At the same time, there was considerable concern about raising premature hopes that such a demonstration would herald an effective cure for EB.

The discussion about the specifics of an ideal gene therapy trial concentrated on various technical aspects, including the type of mutation (nonsense *vs* missense), the use of an *in vivo vs ex vivo* approach for the gene transfer, targeting the epidermal stem cell population, the extent of the follow-up, etc. The importance of such experimental details on the overall outcome is currently uncertain, in part due to the lack of precise information on certain aspects of the technologies used for gene transfer in general. A particularly troublesome issue identified relates to the methodologies to be used to measure the outcome of the corrective efforts. Specifically, routine histopathologic, ultrastructural, and immunofluorescence analyses can readily be performed on EB skin, but how they relate to the functional aspects of the skin remains unclear. Collectively, the discussion concluded that a carefully controlled multicenter approach to initiate gene therapy trials for EB should be embraced, and the details should be left to a working committee in coordination with DEBRA International. It was also emphasized that these studies should take advantage of the National Vector Laboratory, as well as utilize the expertise of the private industry in a collaborative manner.

On the basis of the considerations summarized above, the following recommendations were made:

- 1** Development of an international multicenter pilot project to demonstrate the feasibility (“proof of principle”) of gene therapy in EB. The details regarding construct design, delivery mode, target mutation, assessment of the risk/benefit ratio, etc. should be evaluated by a working committee coordinated by the DEBRA International.
- 2** Establishment of criteria and technologies suitable for objective, reproducible, and clinically relevant assessment of the efficacy of the corrective gene transfer. This could include methods evaluating skin at the DNA, mRNA and protein levels, as well as ultrastructural and functional assays.
- 3** Evaluation of the usefulness of existing animal models as a target of gene therapy in EB, and development of additional animal models with novel technologies allowing temporally conditional and tissue-specific expression of the gene defect.
- 4** Adoption of general guidelines and ethical principles for gene therapy trials in EB, with particular concern of the expectations of the outcome among this patient group.

SUMMARY AND OVERALL RECOMMENDATIONS

It was recognized that the three major clinical challenges addressed by this Symposium, i.e., wound healing, carcinogenesis, and gene therapy, when considered in the context of EB, form three partially overlapping spheres. Outside the overlap, a tremendous amount of research is being conducted, both by academic and commercial groups, and some of this research could, with appropriate extrapolation, be directly applicable to EB. It was recognized that implementation of the consensus plans developed in this visioning meeting requires international collaborative efforts, with the guidance and support of DEBRA International, with strong additional funding from national and international sources, including the National Institutes of Health, the Medical Research Council (U.K.), the Wellcome Trust, the Dermatology Foundation, and others.

In addition to specific recommendations in the areas of research on wound healing, carcinogenesis, and gene therapy in EB, as outlined above, the following overall recommendations on research and clinical emphasis on EB, with the leadership and guidance of DEBRA International were made:

- 1 Sharing of expertise and information among the researchers working on EB and related skin diseases. This could be facilitated by development of an on-line central registry of scientists and clinicians with interest in EB, listing their current research priorities, skills, and specialized technologies available.
- 2 Fostering of global exchange of information and scientific resources, including clinical material, to promote research in EB. This includes organization of workshops and symposia to evaluate periodically the state of research on EB.
- 3 Continued efforts to ensure stability of funding in support of research in basic cutaneous biology and on cutaneous diseases, as exemplified by EB.

- 4 Continuous and close interactions of dermatologic investigators and clinicians with DEBRA International, the coalition of EB patient advocacy organizations around the world.

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